SUPPLEMENTAL MATERIAL

Chemical Measurement Methods

Overview:

There were five daily $PM_{0.25}$ particle filters collected before each weekly blood draw for biomarkers. These five filters were sectioned and then the filters were composited and extracted as a single composite sample. The extractions and digestions used for the analysis of the particulate matter filters described below are specifically designed to dissolve or in the case of metals, digest, the target analytes such that the insolubility of target species for GCMS, ICPMS, and IC analysis is not an issue. The only analysis method where solubility is an issue is water soluble organic carbon (WSOC), which is specifically designed to only analyze the soluble fraction. These WSOC were filtered after extraction to remove non-soluble organic carbon before analysis.

Organic speciation analysis:

As discussed in the text of the paper, PM samples were collected onto filter substrates using the Sioutas^{TM} Personal Cascade Impactor Sampler (PCIS) (SKC Inc, Eighty Four, PA). Losses may be possible since our filter samplers are not designed to guarantee preservation of labile species; although we have not conducted a comprehensive evaluation of these losses, as it was behind the scope of our work, the PCIS impactors operate with a much lower pressure drop (i.e. 10 inches H_2O) than typical FRM samplers and as result these losses should be lower than the FRM method used by EPA, federal and state districts to monitor air quality and other filter based sampling methods.

The methods of analysis to quantify individual organic compounds in the collected aerosol samples are based on earlier established solvent extraction methods (Sheesley et al. 2004). Key compounds used in the epidemiologic analyses are listed in Table 1 below. Procedures for sample extraction and molecular quantification for the organic tracers have been described in detail by Phuleria et al. (2006) and only a brief summary is presented here. The filter samples are spiked with known amounts of isotope labeled internal standard compounds, including three deuterated PAH, three deuterated alkanoic acids, four deuterated alkanes, deuterated cholestane, deuterated cholesterol, and deuterated levoglucosan. Samples are extracted in dichloromethane and methanol, combined, and reduced in volume to 100-250 □L by rotary evaporation followed by pure nitrogen evaporation. The final target volume is determined based on the amount of organic carbon mass in each sample (Phuleria et al. 2006). The extracts are derivatized using diazomethane to convert organic acids to their methyl esters and run on the GCMS. An aliquot of the sample extract is then silylated and run on the GC-PCI-MS to measure levoglucosan and other polar organic compounds (Lewandowski et al. 2008; Stone et. al., 2009).

The methylated and silylated samples are analyzed by auto-injection into a GC/MSD system (GC model 5890, MSD model 5973, Agilent). A 30 m × 0.25 mm DB-5MS capillary column (Agilent) is used with a splitless injection. Along with the samples, six dilutions of authentic quantification standard solutions are also injected and used to determine calibration curves for the compounds of interest. While some compounds are quantified based on the response of a matching compound in the standard mixtures, others for which matching standards are not available are quantified using the response factors of compounds with similar structures and retention times.

Field blanks, laboratory blanks, spiked samples, and small aliquots of standard reference material (NIST Urban Dust SRM 1649a) were analyzed along with the composite

$PM_{0.25}$ samples used for organic tracer compound analysis by GC/MS. Analytical errors for these methods were calculated by compound using spike recovery and the standard deviation of blank filter analysis. All measurements were blank corrected using the average and standard deviation of the blanks. Point-wise estimates of uncertainties for each measurement, which were based on analytical uncertainties and uncertainties associated with blank correction, were used to determine if each measurement are statistically different from zero. Although duplicate samples were not available to evaluate method precision based on our experimental protocol of the study, the precision of the spike and standard reference material analyses were used to estimate method precision.

Elemental analysis:

This was performed on sections of the Teflon filter by means of Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) using a microwave-assisted digestion that uses a mixed acid (Lough et al. 2005). The complete dissolution of metals present in aerosols is achieved by microwave-assisted acid digestion in Teflon bombs. An automated, temperature and pressure-regulated, trace analysis microwave system (Milestone Ethos+) was utilized. The acid chemistry employs a mix of ultra-high purity acids (0.6 mL 16N HNO3, 0.2 mL 12N HCl, 0.1 mL 28N HF). A typical 36-sample batch consists of 22 unknowns, 6 standard reference materials (SRMs), 4 matrix blanks, 2 method blanks, and 2 matrix spikes. The SRMs used to monitor digestion performance were selected to characterize phases that represent actual aerosols or significant aerosol components. These included the NIST SRMs: Recycled Auto Catalyst (#2556), Urban Dust (#1649a), and San Joaquin Soil (#2709). University of Wisconsin-Madison's ICPMS facilities (JJ Schauer) include two quadrupole-based ICP-MS instruments, and one magnetic-sector high-resolution ICP-MS system, all installed in a dedicated trace metal clean room. Instrumental detection limits are 2-3 orders of magnitude lower than with XRF systems.

Water Soluble Organic Carbon (WSOC):

SOA is comprised of polar and highly oxygenated compounds that as a result are water soluble. Therefore, WSOC will capture the presence of these components on particle extracts. WSOC was measured by extracting sections of the Teflon filters in high purity water and then performing the analysis by a Shimadzu TOC-V CSH/CSN Total Organic Carbon Analyzer (Zhang et al. 2008). WSOC was quantified in this method by a non-dispersive infrared detector after catalytic conversion of OC to CO_2 at 680 °C. Inorganic carbon in the water extracts was eliminated prior to analysis by acidification (HCl) and sparging (zero-air). Calibration curves (potassium hydrogen phthalate) followed by two MQ blanks were used every 10 samples. Analytical precision for this method typically falls in the range of 1-4% RSD.

References

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Table 1. Selected organic components measured in $PM_{0.25}$.

Low molecular weight PAH (2-3 ring)	**Hopanes**	**n-Alkanes**
Phenanthrene	17α(H)-22,29,30-Trisnorhopane	n-Tetracosane
Anthracene	17β(H)-21A(H)-30-Norhopane	n-Pentacosane
Fluoranthene	17α (H)-21β (H)-Hopane	n-Hexacosane
Acephenanthrylene	22S-Homohopane	n-Heptacosane
Pyrene	22R-Homohopane	n-Octacosane
9-Methylanthracene	22S-Bishomohopane	n-Nonacosane
Benzo(ghi)fluoranthene	22R-Bishomohopane	n-Triacontane
Cyclopenta(cd)pyrene	22S-Trishomohopane	n-Hentriacontane
Benz(a)anthracene	22R-Trishomohopane	n-Dotriacontane
Chrysene		n-Tritriacontane
1-Methylchrysene	**Selected organic acids**	n-Tetratriacontane
Retene	n-Octanoic acid	n-Pentatriacontane
	n-Decanoic acid	n-Hexatriacontane
Medium molecular weight PAH (4 ring)	n-Dodecanoic acid	n-Heptatriacontane
Benzo(b)fluoranthene	n-Tetradecanoic acid	n-Octatriacontane
Benzo(k)fluoranthene	n-Pentadecanoic acid	n-Nonatriacontane
Benzo(j)fluoranthene	n-Hexadecanoic acid	n-Tetracontane
Benzo(e)pyrene	n-Heptadecanoic acid	
Benzo(a)pyrene	n-Octadecanoic acid	
Perylene	Palmitoleic acid	
	Oleic acid	
High molecular weight PAH (5-6 ring)	Phthalic acid	
Indeno(1,2,3-cd)pyrene		
Benzo(ghi)perylene		
Dibenz(ah)anthracene		
Picene		
Coronene		
Dibenzo(ae)pyrene		

Chemical mass balance (CMB) model

We present more details for the estimation methods used in developing the CMB model as well as results in Arhami et al. (in press). We used a CMB model developed by the US Environmental Protection Agency (version CMB8.2) to apportion the total measured organic carbon (OC) to various key sources of $PM_{0.25}$. We made an *a priori* selection of OC emission sources relevant to the study area based on data from previous studies. They included vehicular traffic (Kuhn et al. 2005; Ntziachristos et al. 2007b; Phuleria et al. 2007), ocean vessels (Agrawal et al. 2008; Rogge et al. 1997) and biomass burning (Fine et al., 2004). To estimate summed emissions from a mixture of vehicular sources including light duty and heavy-duty vehicles we used vehicular profiles corresponding to roadway data from studies we carried out along the CA-110 and I-710 freeways in Los Angeles (Phuleria et al. 2007). In model testing we found other primarily indoor OC sources were not quantifiable (meat cooking and natural gas) or their contribution was too low (<1% of OC), including candle smoke and cigarette smoke (all communities and recruited subjects were nonsmoking).

We chose a set of fitting species for OC emission sources from GC/MS and HR-ICPMS tracer compounds based on chemical stability (Schauer et al., 1996), available data on their concentrations in different source profiles and in ambient data, and previous studies that identified markers for the chosen sources (Schauer et al., 1996; Simoneit, 1999; Schauer and Cass, 2000). We used the following organic compounds and elements as fitting species: EC, Benzo(k)fluoranthene, Benzo(e)pyrene, Benzo(b)fluoranthene, Benzo(ghi)perylene, Coronene, 17□(H)-22,29,30-Trisnorhopane, 17□(H)-21□(H)-Hopane, 22S-Homohopane, 22R-Homohopane, Sitostane, Levoglucosan, Vanadium and Nickel. They were used only for emissions from primary sources of OC (vehicles, ships, and biomass burning). We also estimated other contributions to $PM_{0.25}$ including sulfate, sea spray, resuspended dust, and secondary organic aerosols (SOA), which was estimated from measurements of water soluble organic carbon (WSOC).

It is important to note that SOA in the study region of Los Angeles is presumed to be largely formed from photochemical reactions involving biogenic and anthropogenic volatile organic compounds that react with oxidants such as O_3, OH, and NO_3 radicals to produce products with low volatility including the organic acids measured here (Table 1 above). These products then condense onto existing particles especially in the accumulation mode (Fine et al. 2008). $PM_{0.25}$ consists of some fraction of such accumulation mode particles since it is not purely an ultrafine size cut.

Weekly source contributions were estimated using the CMB model. CMB model fit was evaluated from regression parameters for each week's sample. Variance explained (R^2) was within the desired ranges (0.81-1.00). The χ^2, or weighted sum of squares of the differences between the calculated and measured fitting species concentrations, was also within the desired ranges (0.0-5.7).

Source apportionment results by season and indoor-outdoor locations are shown in Figure 1 using our data from Arhami et al. (in press). Vehicular sources showed the highest contribution to measured outdoor $PM_{0.25}$ mass (35%). Estimated SOA accounted for only 10% of outdoor $PM_{0.25}$. The average outdoor SOA contribution in the warmer phase was somewhat higher (12%) than during the colder phase (8%), which is generally expected given role of photochemical oxidation in SOA generation. Resuspended dust contributions were 9% of outdoor $PM_{0.25}$. The main outdoor source of resuspended dust in the LA region is road dust. The contribution of biomass burning to $PM_{0.25}$ was relatively low (2%). Sea spray and ship emission contributions were negligible because the study sites are located far from the ocean.

Relative contributions from the various for sources were similar for the indoor environment. The average un-apportioned fraction of $PM_{0.25}$ was 33 ± 15%, with larger unknown sources indoors and during the cool season (Figure 1). A sizeable fraction of this un-apportioned outdoor mass is likely attributable to ammonium nitrate, which was not measured since we did not perform ion chromatography on the collected filter samples. The contribution of vehicular sources to indoor $PM_{0.25}$ mass was similar to outdoor data as reflected by I/O ratios close to 1.0. This signifies an important contribution of primary combustion aerosol components from traffic to indoor exposures where people spend a majority of their time. However, SOA concentrations were higher indoors than outdoors in the cool season and this was reversed in the warm season. This might be due in part to chemical reactions of household products with oxidants and hydroxyl radicals leading to the formation of secondary aerosols (Weschler and Nazaroff, 2008), which in the winter may be higher due to lower indoor-outdoor air exchange.

We anticipated regional differences across the four retirement communities studied in the Los Angeles air basin. This was reported elsewhere (Arhami et al. in press). Three communities were in the San Gabriel Valley, usually downwind of downtown Los Angeles most days, and one in Riverside County, much further downwind and closer to inland deserts. PAHs were lowest in Riverside (outdoor, 0.54 ng/m^3) compared with the three San Gabriel Valley sites (1.06 ng/m^3), which were closer to heavy traffic sources in Los Angeles (Arhami et al. in press).

References

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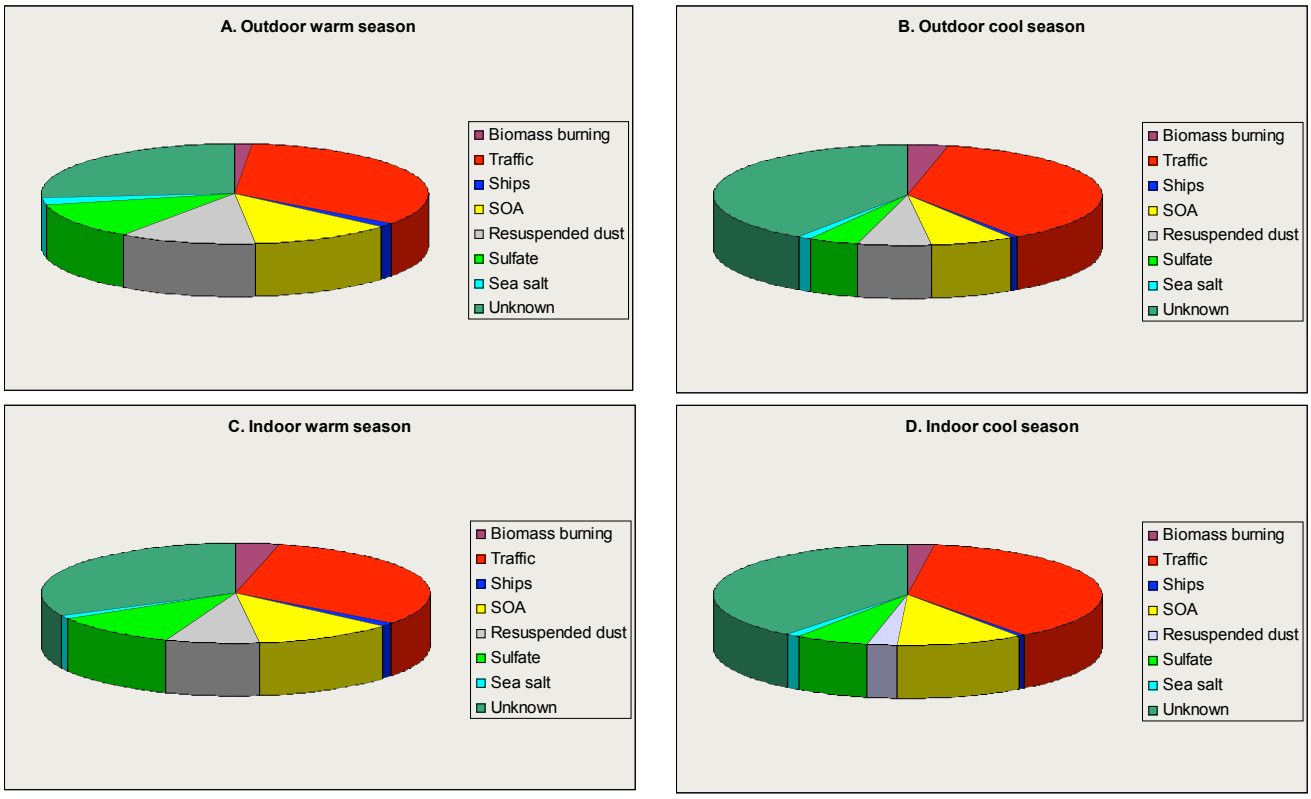
Figure 1. Source contributions to quasi-ultrafine PM by indoor-outdoor sampling site and season.[a] (A) Outdoor warm season, (B) Outdoor cool season, (C) Indoor warm season, (D) Indoor cool season.



[a] Adapted from Arhami et al (Indoor Air, in press).

Regression Model, Mean Centering Method

The following was previously presented in Environmental Health Perspective (Delfino et al. 2009) and is presented again here for consistency. Group outdoor community exposures are assigned to each subject in each of their two seasonal phases of study. Thus, there are three different exposure-outcome relationships that will affect the interpretation of air pollutant associations with a subject's biomarkers: 1) the between-group effect; 2) the within-group, between-phase effect; and 3) the within-subject, within-phase effect. The between-group effect of exposure is the overall biomarker concentrations associated with differences in the air pollutants across groups. This is potentially confounded by time-independent group characteristics, such as the cultural practices, diet, or health-related activities in the retirement community that could affect biomarkers. The within-group, between-phase effect of exposures effect is the overall biomarker concentrations associated with differences in the air pollutants across seasonal phases for the same group. Because the phases are at different periods, this exposure effect may be confounded by other unmeasured seasonal factors. The within-phase, within-subject effect of exposure is the parameter of interest. This is the association of overall biomarker concentrations with differences in the air pollutants across weekly measurements in the same phase for the same subject.

The following mixed model was tested as proposed by Janes et al. (2008):

Let the index *i* indicate the retirement community group (*i* = 1,2,3,4), *j* indicate season (phase) within year 1 and 2 (*j* = 1,2,3,4) nested within community, *k* indicate subject (*k* = 1,…,60) within community, and *t* indicate the weekly biomarker measurement (*t* = 1,…,12). Then a given biomarker measurement, $Y_{i,j,k,t}$ will be related to the following three different exposure-outcome relationships:

$\overline{X}_{ik}$ is the between-group (*bg*) component, which is the average exposure for group *i* assigned to each subject *k*, and

$\overline{X}_{ijk} - \overline{X}_{ik}$ is the within-group, between-phase (*wgbp*) component for subject *k* in group *i*, or the average exposure in phase *j* minus the overall average exposure.

We're still most interested in associations for within-phase exposures assigned to each subject in the group as follows:

$X_{ijkt} - \overline{X}_{ijk}$ is the within-subject, within-phase (*wswp*) component, which is the assigned exposure at biomarker measurement time *t* for subject *k* minus the average exposure for the phase.

The mixed model is then:

$$Y_{i,j,k,t} = a_{i,j,t} + \alpha Z_{i,jk,t} + \beta_{bg}\overline{X}_{ik} + \beta_{wgbp}\left(\overline{X}_{ijk} - \overline{X}_{ik}\right) + \beta_{wswp}\left(X_{ijkt} - \overline{X}_{ijk}\right) + \varepsilon_{i,j,k,t}$$

Where $a_{i,j,k}$ is the random subject intercept nested in group and phase, $Z_{i,j,k}$ is a vector of specifying covariates such as temperature that could change over time, and $\varepsilon_{i,j,k,t}$ denotes random within-person error in the biomarker measurement.

References

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Table 2. Associations of biomarkers of systemic effect with indoor and outdoor 5-day average $PM_{0.25}$ transition metals, regression coefficient (95% CI).[a]

Air Pollutant	IL-6 (pg/mL)	TNF-RII (pg/mL)
V		
Indoor	-0.19 (-0.42, 0.03)	34 (-69, 137)
Outdoor	-0.22 (-0.40, -0.03)*	-19 (-106, 69)
Cr		
Indoor	-0.001(-0.006, 0.004)	-1 (-3, 2)
Outdoor	0.001 (-0.004, 0.01)	-0.4 (-3, 2)
Mn		
Indoor	-0.001 (-0.06, 0.06)	-12 (-47, 23)
Outdoor	0.04 (-0.05, 0.12)	-11 (-57, 35)
Fe		
Indoor	0.01 (-0.02, 0.04)	-5 (-24, 14)
Outdoor	0.04 (-0.05, 0.14)	-10 (-63, 42)
Ni		
Indoor	-0.002 (-0.01, 0.01)	-2 (-7, 4)
Outdoor	0.003 (-0.009, 0.01)	-1 (-7, 6)
Cu		
Indoor	-0.04 (-0.10, 0.02)	-10 (-41, 20)
Outdoor	0.02 (-0.09, 0.13)	-22 (-82, 38)
Zn		
Indoor	0.10 (-0.05, 0.26)	26 (-54, 107)
Outdoor	0.03 (-0.12, 0.19)	-4 (-86, 77)

* $p < 0.05$

[a] Regression coefficients and 95% confidence intervals are for the expected change in the biomarker among 60 subjects associated with an interquartile range change in the air pollutant (see Table 2 of manuscript), adjusted for temperature.

Table 3. Associations of erythrocyte glutathione peroxidase-1 and Cu,Zn superoxide dismutase with indoor and outdoor $PM_{0.25}$ mass and organic components,[a] among all subjects and subjects with positive and negative responses.[b]

	All 60 Subjects		$PM_{0.25}$ responders		$PM_{0.25}$ responders	
Air Pollutant	**SOD (U/g Hb)[c]**	**GPx-1 (U/g Hb)[c]**	**SOD (U/g Hb)**	**GPx-1 (U/g Hb)**	**SOD (U/g Hb)**	**GPx-1 (U/g Hb)**
$PM_{0.25}$ Mass Indoor	-95 (-249, 59)	-0.64 (-1.28, 0.01)	1009 (-165, 2183)	2.27 (-0.76, 5.29)	-206 (-332, -80)*	-0.85 (-1.51, -0.19)*
Outdoor	-16 (-303, 271)	-0.23 (-1.44, 0.97)	2995 (938, 5051)**	6.89 (1.29, 12.50)*	-198 (-437, 40)	-0.59 (-1.81, 0.64)
WSOC Indoor	-67 (-233, 98)	-0.41 (-1.11, 0.30)	684 (-641, 2009)	0.58 (-3.20, 4.35)	-131 (-269, 7)	-0.47 (-1.19, 0.24)
Outdoor	15 (-133, 162)	0.17 (-0.47, 0.81)	33 (-1186, 1252)	3.02 (0.05, 5.99)*	23 (-103, 149)	0.15 (-0.51, 0.80)
PAH total Indoor	-168 (-348, 12)	-0.56 (-1.29, 0.16)	574 (-526, 1674)	0.88 (-2.53, 4.28)	-258(-404, -113)**	-0.73 (-1.47, 0.02)
Outdoor	-94 (-251, 63)	-0.33 (-0.98, 0.33)	1632 (-35, 3299)	3.23 (-1.38, 7.85)	-165 (-291, -39)*	-0.44 (-1.09, 0.22)
PAH LMW Indoor	-103 (-304, 98)	-0.01 (-0.83, 0.82)	1162 (-134, 2457)	2.34 (-1.73, 6.40)	-230 (-395, -65)**	-0.19 (-1.03, 0.65)
Outdoor	-29 (-186, 128)	-0.03 (-0.69, 0.63)	1956 (528, 3384)**	5.27 (0.95, 9.59)*	-112 (-240, 17)	-0.15 (-0.81, 0.51)
PAH MMW Indoor	-147 (-350, 55)	-0.76 (-1.58, 0.05)	873 (-312, 2058)	2.04 (-1.53, 5.61)	-290(-454, -125)**	-1.02 (-1.85, -0.18)*
Outdoor	-101 (-269, 67)	-0.51 (-1.21, 0.19)	1700 (264, 3136)*	3.51 (-0.18, 7.20)	-217(-354, -81)**	-0.69 (-1.39, 0.02)
PAH HMW Indoor	-193 (-350, -36)*	-0.55 (-1.19, 0.08)	125 (-849, 1099)	-0.50 (-3.56, 2.57)	-224 (-352, -95)*	-0.63 (-1.29, 0.02)
Outdoor	-175 (-346, -4)*	-0.41 (-1.12, 0.31)	-153 (-2136, 1830)	-1.51 (-7.47, 4.46)	-160 (-298, -22)**	-0.42 (-1.13, 0.30)
Hopanes Indoor	-91 (-261, 79)	-0.06 (-0.76, 0.64)	446 (-642, 1534)	3.85 (0.54, 7.16)*	-172 (-313, -31)*	-0.29 (-1.00, 0.42)
Outdoor	-36 (-150, 77)	0.27 (-0.22, 0.76)	195 (-350, 739)	0.78 (-0.90, 2.47)	-124 (-228, -20)*	0.20 (-0.32, 0.71)
n-Alkanes Indoor	-2 (-40, 37)	-0.04 (-0.20, 0.12)	-1542 (-4547, 1463)	0.17 (-0.79, 1.13)	0.03 (-31, 31)	-0.05 (-0.22, 0.11)
Outdoor	28 (-7, 63)	-0.05 (-0.19, 0.10)	244 (-1370, 1857)	0.56 (-3.95, 5.07)	25 (-3, 52)	-0.05 (-0.19, 0.09)
Organic Acids Indoor	-81 (-212, 50)	0.04 (-0.58, 0.66)	-562 (-1415, 291)	2.04 (0.30, 3.78)*	-44 (-169, 82)	-0.02 (-0.66, 0.62)
Outdoor	-62 (-200, 77)	0.04 (-0.57, 0.65)	-1174 (-2041, -307)*	-2.97 (-6.02, 0.07)	58 (-64, 180)	0.24 (-0.37, 0.86)

$PM_{0.25}$: particulate matter < 0.25 µm; WSOC: water soluble organic carbon; PAH: polycyclic aromatic hydrocarbons; LMW: low molecular weight (2-3 ring); MMW: medium molecular weight (4 ring); HMW: high molecular weight (> 4 ring).

* $p < 0.05$, ** $p < 0.01$

[a] Regression coefficients and 95% confidence intervals are for the expected change in the biomarker associated with an interquartile range change in the air pollutant (see Table 2 of manuscript), adjusted for temperature.

[b] The models of positive responders to $PM_{0.25}$ presented include data from five subjects for Cu,Zn SOD and from three subjects for GPx-1. One subject was a positive responder for both biomarkers. Models of negative responders to $PM_{0.25}$ are restricted to 55 subjects for Cu,Zn-SOD and 57 subjects for GPx-1. Responder groups were previously defined based primarily on outdoor $PM_{0.25}$ mass, including shorter averaging times, and other exposures such as elemental carbon (Delfino et al. 2009).

[c] Thawed erythrocyte lysates were assayed spectrophotometrically for activities of the antioxidant enzymes copper, zinc-superoxide dismutase (Cu,Zn-SOD) and glutathione peroxidase-1 (GPx-1) (Cayman Chemical, Ann Arbor, MI). Cu,Zn-SOD and GPx-1 values were normalized to units per gram of hemoglobin (U/g Hb). See Delfino et al. (2009) for further details and discussion of the methods and related results.

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